Posters

New Methods for Studying Dynamics in Macromolecules

2309-Pos Board B1
Development of a High Throughput X-Ray Footprinting Facility at the Advanced Light Source to Study the Structure and Dynamics of Complex Biological Macromolecules
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Radiolytic footprinting is an increasingly popular method for structural elucidation of macromolecules in the solution state. X-ray footprinting in particular has developed in the past decade into a nearly routine technique and had been applied to a diverse range of biological systems, yielding unique structural insights impossible to obtain using the more standard structural methods of crystallography, NMR, electron microscopy and small angle scattering. The technique was pioneered at the National Synchrotron Light Source (NSLS) over the past decade; now with the user community continuing to grow and the anticipated closure of this facility, it is imperative that x-ray footprinting continue to be developed. Towards this end we are building a new footprinting beamline at the Advanced Light Source (ALS) synchrotron which will not only support the NSLS users during the commissioning of the NSLS-II, but will provide unique capabilities for the continued development of the method. In particular, our preliminary data show that flux densities produced by an ALS focused white-light bend magnet beamline are high enough to allow microsecond exposures yielding sufficient modification to conduct footprinting experiments. In addition, in-house mass spectrometry availability and expertise will allow the installation of a mass spectrometer instrumentation directly at the beamline. Combined with commercially available tools for automated sample handling, rapid sample digestion and data processing methods, this will allow rapid screening of a large number of samples and will be especially important for samples sensitive to degradation. The high-throughput methodology and microsecond regime made available at the ALS beamline will be highly complementary to the planned NSLS-II footprinting beamline, and both resources will be necessary to accommodate the growing national user community.

2310-Pos Board B2
Synchrotron X-Ray Footprinting on Tour
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Hydroxyl-radical mediated synchrotron X-ray Footprinting (XF) is a powerful solution-state technique in structural biology for study of macromolecular structure and dynamics of proteins and nucleic acids. Currently, however, only a single facility in the US, National Synchrotron Light Source (NSLS) beamline X28C, is dedicated to its use and development. With the expected shutdown of the NSLS in late 2014, this beamline will no longer be available. Although a new specialized facility for XF (the ''XFP'' beamline) is being constructed at NSLS-II as rapidly as possible, for XF science to continue uninterrupted, footprinting programs must also be pursued at other synchrotron facilities. In order to investigate the potential of XF as a mobile beamline-flexible program, we have performed XF experiments using a benchmark sample at various beamlines at APS, CHESS and ALS. We have found that each facility is capable of supporting XF experiments in a general user mode using a simple and transportable flow cell apparatus and provide a description of relevant capabilities for each beamline (including beam parameters, sample dose rates, ease of access, ease of setup, and availability of support). In the course of these experiments, we have also demonstrated the importance of X-ray flux density via comparison of the effects of different lengths of X-ray exposure (with the same overall X-ray dose) on the data quality. Although more complex samples will continue to require high flux density X-rays in order to provide high-quality signal-to-noise data in strongly radical-scavenging environments, many experiments may be performed with more relaxed X-ray beam requirements at less specialized facilities. The success of these experiments indicates that accessibility of synchrotron X-ray footprinting can be broadened significantly with minimal equipment requirements, opening the way for this technique to be available at multiple facilities around the US.

2311-Pos Board B3
Development of a Flexible Platform for Studying the Single Molecule Dynamics of Nucleic Acid Manipulating Enzymes by TIRF Microscopy
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Proper replication and maintenance of genomic material is fundamental to all life. Nature utilizes a large array of complex molecular machines to physically manipulate DNA, manipulations that are accomplished by mechanisms ranging from small and subtle shifts in protein structure, to large and dynamic changes in oligomerization and dissociation events between protein domains and even entire enzymatic assemblies. Two distinct examples from the later end of this spectrum are DnaA, the bacterial replication initiator responsible for unwinding and melting origin DNA, and eukaryotic topoisomerase II, which aids in maintaining genomic integrity and in decatenating daughter chromosomes by modulating the super-helical structure of DNA. Current structural and biochemical data support both large scale dynamic mechanisms for the action of DnaA and topoisomerase II, and further suggest labeling strategies for fluorescent localization and FRET studies by which to characterize these dynamic movements in real time. To this end, we have combined established single molecule TIRF microscopy and solution switching microfluidic chip designs as part of a flexible platform to follow both DnaA assembly with various nucleic acid substrates, including supercoiled plasmids, and the dynamic movements associated with topoisomerase II mediated strand passage. FRET-based assays using either fluorescently labeled DnaA, fluorescently labeled nucleic acid substrates, or both, will serve to address the role of DnaA assembly in its manipulations of double and single-stranded DNA. In the case of topoisomerase II, a set of fluorescently labeled constructs have been designed which track the movements of the enzyme's three protein gates via FRET during the DNA strand passage reaction on supercoiled plasmid. Here, we provide a proof of principle for both systems, and additionally present preliminary data on the dynamics of these two molecular machines.

2312-Pos Board B4
Functional Dynamics of the Packaging Motor P4 Probed by Hydrogen Exchange and Simulation
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The structure and dynamics of large macromolecular assemblies is crucial to the understanding of their biological function but eludes most experimental and computational techniques. One of the promising experimental techniques to follow dynamics is hydrogen-deuterium exchange detected by mass spectrometry. Here we describe a new computational method for quantitative interpretation of deuterium exchange kinetics and validate it using a hexameric viral packaging motor P4. The packaging motor translocates RNA into virus capsid at the expense of ATP hydrolysis. Snapshots of the conformational changes throughout the catalytic cycle have been obtained in atomic details and deuterium exchange data is available, making it a good test model. The prediction of exchange kinetics exploits sampling of structural fluctuations from all atom molecular dynamics simulations on nanosecond time-scale. The resulting residue specific protection factors are used to generate peptide fragment specific exchange kinetics for direct comparison with mass spectrometry data. The predicted kinetics follow remarkably well the experimental data. Our approach is also a powerful tool to validate the assignment of the fragments and to probe ensembles of conformations that cannot be observed by X-ray crystallography, such as the opening of the hexamer.

2313-Pos Board B5
The Blood Plasma Particles Sizes Oscillations Observed by Dynamic Light Scattering
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The human blood plasma is a very complex liquid that contains huge diversity of different molecules (e.g. proteins, peptides, lipids, carbohydrates, etc.) and their multicomponent complexes. It is known that enzymatic degradation processes lead to the particles sizes decreasing and the aggregation ones result in the particles sizes increasing. Due to the equality of the rates of these processes the sizes of particles in the blood plasma in vivo should be constant. After blood sampling the balance of the processes described above can be disturbed and the extent of such disturbance depends on the initial condition of donor's health.